Mycotic Keratitis Due to Curvularia senegalensis and In Vitro Antifungal Susceptibilities of Curvularia spp.

JOSEP GUARRO,1* TIYOMI AKITI,2 ROBERTO ALMADA-HORTA,3 L. A. MORIZOT LEITE-FILHO,4 JOSEPA GENE,1 SUELI FERREIRA-GOMES,3 CARME AGUILAR,1 AND MONTSERRAT ORTONEDA1

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, 43201 Reus, Spain,
and Laboratorio de Micología, Hospital Universitario Clementino Fraga Filho, Universidade Federal do Rio de Janeiro,2 Laboratorio Almada Horta,3 and Instituto Benjamin Constant,4 Rio de Janeiro, Brazil

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A case of mycotic keratitis due to Curvularia senegalensis is reported. This case represents the third known reported infection caused by this rare species. Fungal hyphae were detected in corneal scrapings, and repeated cultures were positive for this fungi. The patient was presumed cured after a corneal transplant and treatment with itraconazole, but the infection recurred and the patient is waiting for a keratoplasty. The in vitro antifungal susceptibilities of the case strain and another 24 strains belonging to seven species of Curvularia were tested for six antifungal agents. With the exception of flucytosine, and occasionally fluconazole, the other drugs assayed (amphotericin B, miconazole, itraconazole, and ketoconazole) were highly effective in vitro.

On 14 August 1997, inflammation recurred in the anterior chamber with pupillary block and hypopyon and hyperemic conjunctiva. Treatment with itraconazole (100 mg twice daily) and systemic corticoids was initiated. By 11 November 1997, the patient had improved substantially, and there was no more inflammation. She was discharged. On 9 December 1997, the patient returned, complaining of visual acuity reduction and, under examination, a uveal tract reaction, which was characterized by precipitates on the posterior surface of the cornea, was observed. Pred-fort and atropine drops were prescribed.

* Corresponding author. Mailing address: Unitat de Microbiologia, Departament de Ciències Mèdiques Bàsiques, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Carrer Sant Llorenç 21, 43201 Reus, Tarragona, Spain. Phone: 34 977759359. Fax: 34 977759322. E-mail: umb@fmcs.urv.es.

FIG. 1. Potassium hydroxide wet preparation of the corneal scrapings. Magnification, ×960.
On 28 January 1998, she had intense conjunctival hyperemia (injection), smarting, and cataract complication, still with uveal reaction. On 17 March 1998, she presented secondary glaucoma by pupil obstruction. She did not come back until 26 June 1998, when she showed a fetid yellowish secretion, a new corneal ulcer in the transplanted cornea, conjunctival injection (or hyperemia), and eyelid edema. A recurrence of the fungus was suspected, and ulcer scrapings were collected for new culture. The patient is still awaiting a new keratoplasty.

Portions of the scrapings obtained on both occasions were inoculated on plates of Sabouraud dextrose agar (with and without penicillin [20 U/ml], streptomycin [40 U/ml], and cycloheximide [0.5 mg/ml]), blood agar, and brain heart infusion agar. On all plates, an apparently identical grayish black mold developed with numerous thick-walled, septate conidia, borne on simple, club-shaped conidiophores. Both conidia and mycelium were dematiaceous. The fungal isolates were referred to the Microbiology Unit of the Rovira i Virgili University in Reus, Spain, for identification and an antifungal susceptibility study.

Morphological study. The isolates were subcultured on potato carrot agar (PCA) and oatmeal agar (OA), and incubated at ca. 25°C in the dark. After 7 days on PCA, the colonies were dark brown and velvety but loose cottony at the center and 60 to 62 mm in diameter. On OA, the macroscopic characteristics of the fungus were similar to those on PCA, but it grew more rapidly, reaching 65 to 68 mm in diameter after 7 days. Sporulation was abundant on both media. Conidiophores usually grew directly on the substrate; they were simple or branched, straight or flexuous, smooth walled, up to 130 μm long and 4 to 6 μm wide (Fig. 2A). Conidia had three to five septa (mostly four septa) and were dark brown, with subhyaline or pale brown cells at each end, ellipsoidal or broadly fusiform, slightly curved, 22 to 31 μm long by 11 to 14 μm wide at the broadest part (Fig. 2B). On the basis of these characteristics, the isolates were identified as *C. senegalensis* (Fig. 3). *Curvularia geniculata*, another species involved in some cases of keratomycosis (6), also has conidia, mostly with four septa, but they are markedly geniculate with the central cell usually very different from the rest (dark brown and swollen). The other reported pathogenic species of *Curvularia*, i.e., *C. brachyspora*, *C. clavata*, *C. lunata*, *C. pallescens*, and *C. verruculosa*, usually have conidia with three septa, which also differ from those of *C. senegalensis* mainly by their color, shape, size, or ornamentation. The two clinical isolates of *C. senegalensis* were kept in the Mycology Laboratory of the Faculty of Medicine in Reus, Spain, for identification and an antifungal susceptibility study.

### Antifungal susceptibility testing.

One of the case isolates (FMR 6319) and four additional isolates of *C. brachyspora*,  

![FIG. 2. C. senegalensis FMR 6319. (A) Conidiophores bearing conidia photographed with phase-contrast optics. Magnification, ×384. (B) Conidia photographed with Nomarski optics. Magnification, ×1,200.](http://jcm.asm.org/)

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>MIC (μg/ml)</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>0.06–32</td>
<td>0.250</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>4–128</td>
<td>16</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Fluocytosine</td>
<td>128–256</td>
<td>256</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.06–32</td>
<td>0.5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.5–16</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Miconazole</td>
<td>0.25–4</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* 50% and 90%, MICs for which 50 and 90% of the isolates tested, respectively.
three of C. *clavata*, four of C. *geniculata*, three of C. *lunata*, four of C. *pallescens*, two of C. *selegalensis*, and four of C. *verruculosa* from very diverse sources were tested to determine their susceptibility to antifungal drugs (Tables 1 and 2). Tests were accomplished by a previously described microdilution method (16) performed, where possible, according to the National Committee for Clinical Laboratory Standards' guidelines for filamentous fungi (14) by using RPMI 1640 medium buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid (MOPS), an inoculum of $4 \times 10^2$ to $4.5 \times 10^4$ CFU/ml, an incubation temperature of 30 or 35°C, reading the results after 2 or 3 days (48 or 72 h), and an additive drug dilution procedure.

The MICs of the six antifungal agents against the clinical isolate (FMR 6319) were as follows: 0.25 μg/ml for amphotericin B, 1 μg/ml for miconazole and ketoconazole, 0.25 μg/ml for itraconazole, 16 μg/ml for fluconazole, and 256 μg/ml for 5-fluorocytosine. This is the greatest number of species of *Curvularia* that has been tested so far in a single study for in vitro antifungal susceptibility. Tables 1 and 2 show the antifungal susceptibility results of the 25 isolates. In contrast to the results of You et al. (22), amphotericin B, itraconazole, miconazole, and ketoconazole were highly effective against almost all the species tested. Generally, C. *pallescens* displayed the highest MICs. These results are in agreement with those of Bent and Kuhn (2), who tested five strains of *Curvularia* spp. obtained from sinus aspirates from allergic fungal sinusitis patients. All five strains were sensitive to amphotericin B, ketoconazole, and nystatin; two strains were sensitive to itraconazole, and all were resistant to fluconazole. In addition, Sutton et al. (20) reported C. *lunata* as sensitive to practically all the antifungals available, with the exception of flucytosine.

However, despite the fact that in vitro resistance to a particular agent does provide valuable information in selecting a proper antifungal agent for treatment, these results should be interpreted with caution because in vitro studies are only limited approximations of the in vivo situations. Studies of correlation of in vitro data with clinical outcome are needed for a more...
definitive evaluation of the predictive value of MICs for filamentous fungi.

The genus *Curvularia* comprises about 30 species. It was widely studied and monographed by Ellis (8, 9) and Sivanesan (19). Its teleomorphs are included in the ascomycete genus *Cochliobolus*. Most of the species are pathogens of grains and plants common to tropical areas. They are also commonly found in agricultural soils (7). *Curvularia* spp. are darkly pigmented fungi with conidia efficiently adapted to aerial dispersal (7). They are, therefore, habitual components of air mycobiota and have a worldwide distribution.

*Curvularia* species had been previously considered non-pathogens or thought to affect humans only rarely, but these fungi are now increasingly being reported to cause human disease (3, 22). Seven species of *Curvularia* have been involved in human infections. They are morphologically very similar with the differences mainly in the conidial features (size, number of septa, shape, and ornamentation) (6). In human pathology, *Curvularia* spp. are frequently associated with allergic sinusitis (12), although several cases of ocular infections such as keratitis without trauma have rarely been reported (5).

*Curvularia brachyspora* was reported more than 20 years ago as the first case. *C. senegalensis* has been involved in two cases of allergic bronchopulmonary disease (3, 22). The management of fungal keratitis topically with 5% pimaricin suspensions, but little was reported on October 14, 2017 by guest http://jcm.asm.org/Downloaded from

## TABLE 2. In vitro susceptibilities of *Curvularia* spp. to six antifungal agents

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates</th>
<th>AMB MIC Range</th>
<th>MON MIC Range</th>
<th>ITRA MIC Range</th>
<th>KETO MIC Range</th>
<th>FLU MIC Range</th>
<th>5-FC MIC Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. brachyspora</em></td>
<td>4</td>
<td>0.29 (0.06–2)</td>
<td>0.84 (0.250–4)</td>
<td>0.70 (0.125–32)</td>
<td>0.84 (0.5–4)</td>
<td>22.62 (4–128)</td>
<td>180.9 (128–256)</td>
</tr>
<tr>
<td><em>C. clavata</em></td>
<td>3</td>
<td>0.31 (0.250–0.5)</td>
<td>1</td>
<td>1–2</td>
<td>0.19 (0.125–0.5)</td>
<td>0.62 (0.5–2)</td>
<td>16</td>
</tr>
<tr>
<td><em>C. geniculata</em></td>
<td>4</td>
<td>0.12 (0.06–0.250)</td>
<td>1.41</td>
<td>0.5–4</td>
<td>0.21 (0.125–0.5)</td>
<td>1</td>
<td>0.5–2</td>
</tr>
<tr>
<td><em>C. lunata</em></td>
<td>3</td>
<td>0.12 (0.06–0.5)</td>
<td>1</td>
<td>1–4</td>
<td>0.41</td>
<td>0.06–8</td>
<td>1</td>
</tr>
<tr>
<td><em>C. pallescens</em></td>
<td>4</td>
<td>0.41 (0.06–32)</td>
<td>1.99</td>
<td>0.55–4</td>
<td>2.82</td>
<td>0.5–16</td>
<td>4.70</td>
</tr>
<tr>
<td><em>C. senegalensis</em></td>
<td>3</td>
<td>0.50 (0.250–2)</td>
<td>1</td>
<td>1–2</td>
<td>0.50</td>
<td>0.25–1</td>
<td>1.18</td>
</tr>
<tr>
<td><em>C. verruculosa</em></td>
<td>4</td>
<td>0.25 (0.125–0.5)</td>
<td>1.41</td>
<td>1–2</td>
<td>0.70</td>
<td>0.5–2</td>
<td>1.18</td>
</tr>
</tbody>
</table>

*Antifungal agents are abbreviated as follows: AMB, amphotericin B; MON, miconazole; ITRA, itraconazole; KETO, ketoconazole; FLU, fluconazole; 5-FC, fluocytosine. Geometric mean MICs (expressed in micrograms per milliliter) are shown.*

REFERENCES